ABSTRACT Properly coordinated defense signaling networks are critical for the fitness of plants. One hub of the defense networks is centered on salicylic acid (SA), which plays a key role in activating disease resistance in plants. However, while a number of genes are known to affect SA-mediated defense, relatively little is known about how these gene interact genetically with each other. Here we exploited the unique defense-sensitized Arabidopsis mutant accelerated cell death (acd) 6-1 to dissect functional relationships among key components in the SA hub. We show that while enhanced disease susceptibility (eds) 1-2 and phytoalexin deficient (pad) 4-1 suppressed acd6-1–conferred small size, cell death, and defense phenotypes, a combination of these two mutations did not incur additive suppression. This suggests that EDS1 and PAD4 act in the same signaling pathway. To further evaluate genetic interactions among SA regulators, we constructed 10 pairwise crosses in the acd6-1 background among mutants defective in: SA INDUCTION-DEFICIENT 2 for SA biosynthesis; AGD2-LIKE DEFENSE 1, EDS5, and PAD4 for SA accumulation; and NONEXPRESSOR OF PR GENES 1 for SA signaling. Systematic analysis of the triple mutants based on their suppression of acd6-1–conferred phenotypes revealed complex and interactive genetic relationships among the tested SA genes. Our results suggest a more comprehensive view of the gene networks governing SA function and provide a framework for further interrogation of the important roles of SA and possibly other signaling molecules in regulating plant disease resistance.

IN response to pathogen infection, plants can activate different layers of defense responses and undergo global gene expression reprogramming (Maleck et al. 2000; Tao et al. 2003; Katagiri 2004). A major challenge of the postgenomic era is to identify genes that control plant innate immunity and to elucidate how they are organized into networks to orchestrate host defense responses.

One key hub in plant defense signaling networks is centered on the small phenolic molecule salicylic acid (SA). SA is important for basal defense, resistance protein-mediated defense, and systemic acquired resistance (Hammond-Kosack and Jones 1996; Ryals et al. 1996; Tsuda et al. 2008). The SA hub of Arabidopsis includes many genes, which can be further grouped into three types on the basis of how they affect SA-mediated defense (Lu 2009). Type I SA genes encode enzymes that are directly involved in SA biosynthesis. One example is SA INDUCTION-DEFICIENT 2/ENHANCED DISEASE SUSCEPTIBILITY 16 (SID2/EDS16), which encodes isochorismate synthase contributing to bulk SA biosynthesis (Wildermuth et al. 2001). Type II SA genes encode proteins that do not act directly as SA biosynthetic enzymes. Mutations in these genes lead to partially compromised SA accumulation and enhanced disease susceptibility to pathogen infection, which can be rescued by exogenous SA treatment. The precise mechanism of action for each type II SA gene, however, still remains to be resolved. Examples of type II SA genes include ACCELERATED CELL DEATH 6 (ACD6), AGD2-LIKE DEFENSE 1 (ALD1), EDS1, PHYTOALEXIN DEFICIENT 4 (PAD4), SID1/EDS5, HOPW1-1-INTERACTING 3/AVRPHPB SUSCEPTIBLE 3/GH3-LIKE DEFENSE GENE 1, and the MODIFIER OF SNC1 genes (Falk et al. 1999; Jirage et al. 1999; Nawrath et al. 2002; Lu et al. 2003; Song et al. 2004b; Palma et al. 2005, 2007; Zhang et al. 2005; Zhang and Li 2005; Goritschng et al. 2007; Jagadeeswaran et al. 2007; Lee et al. 2007; Nobuta et al. 2007). Type III SA genes act downstream of SA accumulation. The best-characterized type III SA gene is NONEXPRESSOR OF PR GENES 1 (NPR1), which is the major SA signal transducer (Cao et al. 1997; Ryals et al. 1997; Shah et al. 1997; Dong 2004). Enhanced
disease susceptibility of npr1 mutants to pathogen infection cannot be rescued by SA treatment.

Increasing evidence suggests that defense signaling networks are complex, involving crosstalk to many other signaling pathways (Feyes and Parker 2000; Kunkel and Brooks 2002; Wang et al. 2007; Koonneef and Pieterse 2008; De Torres Zabala et al. 2009) and to plant development (Martinez et al. 2004; Endo et al. 2009; Wang et al. 2011a). A variety of strategies are used to interrogate the topology of defense networks. On the basis of global gene expression profiling, microarray studies have revealed some of the hierarchical structure of components in the SA hub. These studies also showed there are both positive and negative interactions between components in the SA hub and those in ethylene and/or jasmonic acid signaling pathways (Wang et al. 2008; Tsuda et al. 2009; Sato et al. 2010). Analysis of protein complexes has also added further details to defense networks. For instance, type II SA regulators EDS1 and PAD4 were shown to interact physically (Feyes et al. 2001), suggesting that these two proteins function in the same pathway.

An alternative approach to study defense networks is through analyzing mutants with two or more signaling components having been knocked out. This is a classical genetic way to assign genes to specific functional groups and has been widely used in model organisms, such as yeast and Escherichia coli, to understand gene functions and the architecture of signaling networks (Collins et al. 2006, 2007; Roguev et al. 2007; Breslow et al. 2008; Typas et al. 2008).

Arabidopsis is also a premier system for this type of genetic analysis. However, when a mutation in one defense gene already leads to enhanced disease susceptibility, additional susceptible phenotypes caused by mutations in two or more defense genes can be difficult to detect on the basis of standard disease assays. Therefore, more sensitive methods should be developed to assess the functional relationships between certain components on defense networks.

A small Arabidopsis mutant acd6-1 is being used to develop one such method and has already revealed new insights into the SA signaling networks (Song et al. 2004b; Lu et al. 2009). ACD6 is a type II SA regulator that was shown to be a major determinant of fitness in Arabidopsis (Lu et al. 2003; Todesco et al. 2010). acd6-1 is a gain-of-function mutant that demonstrates constitutive defense, severe cell death, and extreme dwarfism (Rate et al. 1999; Vanacker et al. 2001; Lu et al. 2003). The cell death and dwarf phenotypes are sensitized to the change of defense levels in acd6-1. We have taken advantage of this unique feature of acd6-1 in a genetic analysis to understand functional relationships between several SA regulators and in a suppressor screen to identify novel defense genes (Song et al. 2004b; Lu et al. 2009; Wang et al. 2011a,b).

In this article, we exploited acd6-1 in a systematic dissection of functional relationships among components in the SA hub. We showed that while eds1-2 and pad4-1 individually suppressed acd6-1–conferred phenotypes, a combination of these two mutations did not incur additional suppression. Thus, we provided direct genetic evidence to show that EDS1 and PAD4 act in the same signaling pathway. We further conducted a comprehensive evaluation in acd6-1 of pairwise genetic interactions among five SA components: type I SA gene SID2; type II SA genes ALD1, EDS5, and PAD4; and type III SA gene NPR1. Systematic analysis of a total of 10 triple mutants for their defense and cell death phenotypes have revealed complex genetic interactions among the SA genes and suggest interconnected defense signaling networks.

Materials and Methods

Plant materials

All plants used in this report are in Columbia-0 background. Plants were grown in growth chambers with light intensity at 200 μmol m⁻² s⁻¹, 60% humidity, and 22°. The single mutants (acd6-1, ald1-1, npr1-1, pad4-1, eds5-1, and sid2-1), the double mutants (acd6-1ald1-1, acd6-1eds5-1, acd6-1npr1-1, acd6-1pad4-1, and acd6-1sid2-1), and the triple mutants (acd6-1ald1-1pad4-1 and acd6-1npr1-1pad4-1) were previously described (Song et al. 2004b; Lu et al. 2009). eds1-2, which was introgressed to the Col-0 background, was kindly provided by Jane Parker at Max Planck Institute for Plant Breeding Research. eds1-2 was crossed to acd6-1 to make acd6-1eds1-2. Additional triple mutants were constructed by crossing respective double mutants in the acd6-1 background and were screened for homozygotes in the F₂ population using specific derived cleaved amplified polymorphic sequence (dCAPS) markers and/or other polymerase chain reaction (PCR) markers. Genotyping primers used in this study were listed in supporting information, Table S2.

Pseudomonas infection and bacterial growth assay

Pseudomonas infection was performed with 25-day-old plants grown in a chamber with a 12-hr light/12-hr dark cycle. Freshly cultured Pseudomonas syringae pv. maculicola (Pma) ES4326 strain DG3 (OD₆₀₀ = 0.6–0.8) was diluted to a final concentration 1 × 10⁶ cfu ml⁻¹ and infiltrated into the fourth to sixth leaves of each genotype. Three days postinfection, discs of 7 mm diameter from the infected leaves were used for each data point. Statistical analysis was performed with Student’s t-test (StatView 5.0.1).

Cell death staining

For trypsin blue staining, the fourth to sixth leaves of the plants were boiled in lactophenol [phenol:glycerol:lactic acid:water = 1:1:1:1 (v/v)] containing 0.01% trypsin blue for 2 min, cleared off with boiling alcoholic lactophenol (95% ethanol:lactophenol = 2:1), and rinsed with 50% ethanol. The stained leaves were examined with a Stemi SV 1.1 stereomicroscope (Zeiss) and pictures were taken.
with an AxioCam MRc5 camera (Zeiss) connected to the microscope.

**RNA analysis**

Twenty-five-day-old plants were harvested for total RNA extraction with TRIzol reagent (Invitrogen) according to the protocol provided by the manufacturer. Northern blotting was performed as previously described (Lu et al. 2003). Radioactive probe for PR1 was made by PCR with a specific antisense primer for a PR1 fragment in the presence of $[^{32}P]$ dCTP.

**SA measurement**

Twenty-five-day-old plants were harvested for SA extraction followed by HPLC analysis as previously described (Wang et al. 2011a).

**Results**

*acd6-1 is a sensitive tool to detect both additive and nonadditive interactions between SA regulators*

We previously used the defense-sensitized mutant *acd6-1* to elucidate functional relationships between several SA genes (Song et al. 2004b; Lu et al. 2009; Wang et al. 2011a). In this genetic analysis, we introduced two mutations that affect SA-mediated defense, each of which is known to cause suppression of *acd6-1*-conferring phenotypes, into the *acd6-1* background and assessed whether the two mutations together result in additive or nonadditive suppression of *acd6-1*-conferring phenotypes. On the basis of the phenotypes of the triple mutant, we made inferences on the interaction between the respective genes. A nonadditive suppression would indicate that the two genes act in the same pathway, whereas an additive suppression would suggest that the genes function in different pathways. Using this analysis, we demonstrated additive interactions between several SA genes (Song et al. 2004b; Lu et al. 2009; Wang et al. 2011).

To further test the validity of *acd6-1* as an effective tool to detect interactions between defense genes, we set out to analyze in the *acd6-1* background the functional relationship between two previously characterized SA genes, *EDS1* and *PAD4*, that are presumed to act in the same pathway on the basis of biochemical and microarray studies (Feys et al. 2001; Bartsch et al. 2006; Sato et al. 2007). We crossed *acd6-1* with *eds1-2*, which was introgressed to Col-0 by five times of crosses (J. Parker, personal communication). Both *pad4-1* and *eds1-2* partially suppressed *acd6-1*-conferring phenotypes, namely small size, SA accumulation, expression of the defense marker gene *PATHOGENESIS-RELATED GENE 1* (PR1), and constitutive disease resistance to the pathogen *P. syringae* (Figure 1, A–D and Lu et al. 2003). In addition, large patches of cell death in *acd6-1* were also greatly reduced by *eds1-2* (Figure 2). Compared to the two parental double mutants, the triple mutant *acd6-1* *eds1-1* *pad4-1* showed a similar level of suppression of these phenotypes, suggesting a nonadditive interaction between *eds1-2* and *pad4-1*. These results provide direct genetic evidence to demonstrate that *EDS1* and *PAD4* act in the same signaling pathway to regulate *acd6-1*-conferring phenotypes, consistent with evidence from previous studies based on global gene expression profiling and protein–protein interactions (Feys et al. 2001; Bartsch et al. 2006; Sato et al. 2007). Our data also suggest that the *acd6-1* background can be used to unravel both additive and nonadditive genetic interactions among defense genes.

**Multiple SA regulators contribute to both SID2-dependent and -independent defense pathways and/or cell death control**

Next we set out to dissect the genetic interactions among several known SA genes, *SID2* (type I SA gene), *ALD1*, *EDS5*, and *PAD4* (type II SA genes), and *NPR1* (type III SA gene). Loss-of-function mutations in each of these genes partially
suppressed acd6-1–conferred phenotypes (Song et al. 2004b; Lu et al. 2009). We made combinatorial pairwise crosses of these SA mutants in the acd6-1 background and obtained a total of 10 triple mutants, two of which were reported previously (Song et al. 2004b; Lu et al. 2009). Here we performed a systematic analysis of all 10 triple mutants for their defense and cell death phenotypes.

We first examined how type I gene SID2 interacts with type II genes. Consistent with SID2’s major role in SA biosynthesis, we detected only residual SA levels but no expression of the SA marker gene PR1 in acd6-1sid2-1. Compared with acd6-1, acd6-1sid2-1 was larger and had partially reduced resistance to P. syringae infection and cell death (Figures 3–7 and Lu et al. 2009). Since the double mutants acd6-1ald1-1, acd6-1eds5-1, and acd6-1pad4-1 accumulated more SA than acd6-1sid2-1 but less SA than acd6-1, we conclude that these type II SA genes only partially affect SID2-mediated SA biosynthesis (Lu et al. 2009). When each of these type II SA mutations was introduced into acd6-1sid2-1, we detected a small further reduction of glucosyl-conjugated SA (total SA) in the respective triple mutants (Figure 4 and Table S1). These results suggest that type II SA genes regulate both SID2-dependent and SID2-independent SA accumulation and the SID2-independent pathway only plays a minor role in affecting SA accumulation.

Although the effect of SID2-independent pathway(s) on SA accumulation is minor, we observed a strong influence of this pathway on other acd6-1–conferred phenotypes. Compared to the respective double mutants in the acd6-1 background, the triple mutants acd6-1sid2-1ald1-1, acd6-1sid2-1eds5-1, and acd6-1sid2-1pad4-1 exhibited more reduced cell death visible to the naked eye, which was further confirmed by trypan blue staining (Figures 3 and 7). They also had more reduced disease resistance (Figure 6). These results suggest that type II SA genes, ALD1, EDS5, and PAD4, act additively with type I SA gene SID2 in regulating disease resistance and cell death in acd6-1.

Multiple SA regulators contribute to both NPR1-dependent and -independent defense pathways and cell death control

To study how type I and type II SA genes interact with type III SA gene NPR1, we analyzed mutants defective for these genes in the acd6-1npr1-1 background. NPR1, an ankyrin-repeat–containing protein, acts as an SA signal transducer and can also positively or negatively influence SA accumulation (Cao et al. 1997; Ryals et al. 1997; Shah et al. 1997; Dong 2004; Lu et al. 2009). Compared to acd6-1, acd6-1npr1-1 was slightly larger, had reduced PR1 expression, disease resistance, and cell death, but accumulated higher levels of free SA (Figures 3–7 and Lu et al. 2009).

First we examined the interaction between type I gene SID2 and type III SA gene NPR1. The triple mutant acd6-1sid2-1npr1-1 only accumulated residual SA, suggesting that the high SA levels observed in acd6-1npr1-1 are largely produced via the SID2-dependent SA biosynthesis. Compared with the two double mutants, acd6-1sid2-1npr1-1 also displayed further reduced cell death and disease resistance (Figures 3, 6, and 7). While it is possible that one or more SID2-independent pathways are responsible for further

Figure 2  eds1-2 acts nonadditively with pad4-1 in suppressing cell death in acd6-1. The fourth to sixth leaves of the indicated genotypes were stained with trypan blue. Photographs were taken with a dissecting microscope connected to an AxioCam MRC5 camera (Zeiss). eds1-2 and pad4-1 showed no detectable cell death (data not shown). Note the large patches of cell death shown in acd6-1 (arrows) were reduced in the double and triple mutants.

Figure 3  Genetic interactions among SA mutants lead to altered acd6-1 morphology. Plants were photographed 25 days postplanting. The single mutants largely resemble Col-0 (data not shown).
Genetic interactions among SA mutants lead to altered SA accumulation in acd6-1. SA was extracted from 25-day-old plants and analyzed by HPLC for free (A) and total SA (B). Note B has a log scale. The single mutants have similar SA levels as Col-0 (data not shown). Letters indicate significant difference among the samples (P < 0.05).

suppressed disease resistance and cell death in acd6-1npr1-1sid2-1, the additive suppression could also occur if SID2 acts through both NPR1-dependent and -independent pathways.

Next we examined the interaction between type II genes (ALD1, EDS5, and PAD4) and NPR1. The pad4-1 mutant was previously shown to greatly suppress the high SA levels in acd6-1npr1-1. On the basis of this result, we proposed that the negative role of NPR1 in regulating SA levels requires the function of PAD4 (Lu et al. 2009). We confirmed this result in this study. We further observed a similar suppression effect of eds5-1 on SA accumulation in acd6-1npr1-1 (Figure 4), suggesting that NPR1’s negative regulation of SA accumulation also involves EDS5 besides PAD4. Since the SA levels in the triple mutants, acd6-1eds5-1npr1-1 and acd6-1pad4-1npr1-1, were even lower than those in the double mutants, acd6-1eds5-1 and acd6-1pad4-1, we speculate that EDS5 and PAD4 act through both NPR1-dependent and -independent pathways to regulate SA levels. Alternatively, these results can be explained that besides its roles as a positive SA signal transducer and as a negative regulator of SA accumulation, NPR1 plays a positive role in regulating SA accumulation in a separate pathway from those mediated by EDS5 and PAD4. Consistent with reduced SA levels, acd6-1eds5-1npr1-1 and acd6-1pad4-1npr1-1 had much reduced PRI expression, disease resistance, and cell death compared with the respective double mutants (Figures 5–7).

In contrast to acd6-1eds5-1npr1-1 and acd6-1pad4-1npr1-1, the acd6-1ald1-1npr1-1 mutant expressed a high level of PRI transcripts, accumulated similar levels of SA, and displayed similar degrees of disease resistance and cell death as acd6-1ald1-1 or acd6-1npr1-1 (Figures 4–7). These data indicate a nonadditive interaction between ALD1 and NPR1. Given that ALD1 is a type II SA gene and NPR1 is a type III SA gene, we propose that ALD1 acts upstream of NPR1 in the same pathway to regulate plant defense and cell death.

**Genetic analysis reveals additive interactions among type II SA regulators, ALD1, EDS5, and PAD4**

We further analyzed genetic interactions among the three type II SA mutants, ald1-1, eds5-1, and pad4-1 in the acd6-1 background, to learn more about the pathway(s) in which these genes act. Compared with ald1-1 and pad4-1, eds5-1 had a greater suppression of SA levels in acd6-1, suggesting that among these type II SA genes, EDS5 plays a greater role in regulating SA accumulation (Figure 4). This notion is consistent with previous studies (Nawrath and Metraux 1999; Nawrath et al. 2002). When any two of these mutants were genetically combined, we observed further suppression of acd6-1–conferred small size, SA accumulation, PRI expression, disease resistance, and cell death (Figures 3–7 and Song et al. 2004b). These results suggest that type II SA genes do not act in one linear pathway but rather in separate pathways to regulate SA-mediated defense and cell death in acd6-1.

**Discussion**

Genetic analysis directly associates gene functions with phenotypes; thus a genetic approach to study relationships between genes can reveal functional information invisible to other approaches, such as protein–protein interaction and microarray analysis. Here we exploited a sensitive Arabidopsis
mutant \textit{acd6-1}, whose phenotypes (small size and cell death) are easily perturbed by the changes of defense levels, in a genetic interpretation of relationships among several key components in the SA signaling networks. Our data have revealed both additive and nonadditive relationships among these SA genes and suggest highly interactive SA signaling networks (Figure 8).

EDS1 and PAD4 are type II SA regulators that share similarities in their protein sequences. Although the two proteins might have distinct roles in regulating plant defense and other processes (Feys \textit{et al.} 2005; Rietz \textit{et al.} 2011), evidence also suggests that they can act together in the same pathway under certain conditions (Feys \textit{et al.} 2005; Bartsch \textit{et al.} 2006; Sato \textit{et al.} 2007). Our data that \textit{eds1-2} and \textit{pad4-1} act nonadditively to suppress \textit{acd6-1}–conferred phenotypes support the latter notion, suggesting that the two genes function in the same pathway to regulate plant defense and cell death in the \textit{acd6-1} background.

\textit{ALD1} encodes an aminotransferase and was proposed to generate an amino acid-derived signal to activate plant defense (Song \textit{et al.} 2004a). Like \textit{npr1} mutants, \textit{ald1-1} is defective in both local defense and systemic acquired resistance (Song \textit{et al.} 2004b). A nonadditive interaction between the \textit{ald1-1} and \textit{npr1-1} mutations was observed in \textit{acd6-1} and in the syntaxin double mutant \textit{syp121-1syp122-1} (this study and Zhang \textit{et al.} 2007), suggesting that \textit{ALD1} and \textit{NPR1} act in the same branch of a defense pathway.

While our results revealed two cases of nonadditive interactions between SA regulators, the additive interactions appear to be more prevalent. Each SA gene of one type was found to act additively with genes of the other two types. Such additive effects are less likely due to the leakiness of the mutations, since the SA mutants used in this report are generally considered as null mutants (Cao \textit{et al.} 1997; Falk \textit{et al.} 1999; Jirage \textit{et al.} 1999; Wildermuth \textit{et al.} 2001; Nawrath \textit{et al.} 2002; Song \textit{et al.} 2004b). Instead, our results indicate that there are multiple regulatory pathways feeding into the regulation of SA biosynthesis, accumulation, and signaling. Consistent with our results, Tsuda \textit{et al.} (2009)
reported an additive interaction between type I mutant sid2 and type II mutant pad4 in response to bacterial infection and elicitor treatments in the absence of acd6-1.

Interestingly, we found that the degree of suppression of acd6-1–conferred phenotypes by two mutations together is often smaller than the added value from two single mutations. For instance, on the basis of total SA quantification, the degree of suppression of SA accumulation in acd6-1 by ald1-1, eds5-1, and pad4-1 is 52, 94, and 78%, respectively (Table S1). However, the corresponding triple mutants showed 99% reduction in SA levels, a value smaller than any two combined values from above. These observations suggest a negative interaction between most SA genes. The negative interaction can be explained that while most SA genes act in different pathways, they can also functionally compensate each other, possibly due to some genes sharing redundant function and/or they can regulate each other’s function. Indeed, many prior studies showed that expression of some SA genes is dependent on other genes in the SA networks. For instance, expression of ALD1 and EDS5 is known to be PAD4 dependent (Nawrath et al. 2002; Song et al. 2004b) and PAD4 to be NPR1 dependent (Jirage et al. 1999). In addition, expression of some SA genes can also be regulated by SA treatment (Zhou et al. 1998; Falk et al. 1999; Nawrath et al. 2002; Lu et al. 2003; Song et al. 2004b; Jagadeeswaran et al. 2007; Lee et al. 2007). Thus, there are likely interlocked signal amplification loops that involve SA and multiple SA regulators. Consistent with a picture of interactive SA signaling networks, a previous microarray study placed NPR1 both downstream and upstream of type II SA regulators and type I SA gene SID2 (Wang et al. 2008). The highly interactive SA networks suggest that plant innate immunity is robust, involving multiple key components acting in concert to regulate disease resistance to broad-spectrum pathogens.

Understanding how genes function and their interactions with each other to form complex signaling networks governing cellular processes and behavior of organisms has become increasingly important in the postgenomic era. In this report, we have demonstrated the utility of a unique Arabidopsis mutant acd6-1 in elucidating the functional relationships among key components in the SA signaling networks. Together with those results obtained from complementary approaches related to biochemistry and global gene expression profiling, the results from this study have revealed a picture of a complex and interactive genetic map for the SA signaling networks. Therefore, this study provides a framework for further systematic interrogation of the important role of SA and other signaling molecules in plant disease resistance, leading to a better understanding of mechanisms of plant disease resistance.

Acknowledgments

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Literature Cited


Breslow, D. K., D. M. Cameron, S. R. Collins, M. Schuldiner, J. Stewart-Ornstein et al., 2008 A comprehensive strategy en-


Zhang, Y., and X. Li, 2005 A putative nucleoporin 96 Is required for both basal defense and constitutive resistance responses mediated by suppressor of npr1–1, constitutive 1. Plant Cell 17: 1306–1316.

Communicating editor: Craig S. Pikaard
Genetic Dissection of Salicylic Acid-Mediated Defense Signaling Networks in Arabidopsis

Gina Ng, Savanna Seabolt, Chong Zhang, Sasan Salimian, Timley A. Watkins, and Hua Lu
Genetic interactions among SA mutants lead to altered SA accumulation in *acd6-1*.

<table>
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<tr>
<th>Genotypes</th>
<th>Total SA (µg/gFW)</th>
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<td>1 Col</td>
<td>2.3±0.1</td>
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<tr>
<td>8 <em>acd6-1</em></td>
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Total SA value was shown for each genotype. The degree of suppression (S) of total SA accumulation in *acd6-1* by each SA mutant was calculated as following: $S = (\text{SA}_{\text{acd6-1}} - \text{SA}_{\text{double or triple mutant}}) / \text{SA}_{\text{acd6-1}} \times 100$. $\text{SA}_{\text{acd6-1}}$ stands for the total SA value for *acd6-1* and $\text{SA}_{\text{double or triple mutant}}$ stands for the total SA value for a double or a triple mutant in the *acd6-1* background. Statistical analysis was performed with Student’s t-test (StatView 5.0.1). Letters indicate significant difference among the samples ($P<0.05$). The numeric key for the genotypes used in these experiments was the same as that shown in Figures 3-7.
TABLE S2  Primers used in this paper

<table>
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Primer set used to make *PR1* probe for northern blotting

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